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FINAL REPORT

THE DEVELOPMENT OF MOLECULAR MARKERS FOR THE EFFICIENT BREEDING OF HIGH QUALITY, BLACKLEG RESISTANT, YELLOW-SEEDED B. NAPUS

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Project:

The development of molecular markers for the efficient breeding of high quality, blackleg resistant, yellow-seeded *B. napus*.

Abstract

The objective of this project is to develop molecular markers for seed colour, oil content and blackleg resistance in *Brassica napus* canola to speed up the development of high quality, disease resistant varieties of *B. napus* for production on the Canadian prairies. There are two sources of blackleg resistance and two sources of yellow seed colour being examined in the research. The development of molecular markers for quality and disease resistance traits from different sources will allow for efficient selection of progeny lines carrying both sets of genes controlling these traits.

Executive Summary

Under this ADF funded research project the Agriculture and Agri-Food Canada-Saskatoon Research Centre (AAFC-SRC) examined three traits in *B. napus* canola; oil content, seedcoat colour and blackleg resistance. Through improvements in quality and disease resistance, the canola industry continues to create a strong demand for canola oil on domestic and international markets. To facilitate these improvements, a significant amount of plant breeding must be performed to bring these traits to optimum levels in high yielding varieties. To assist plant breeding, this research project has developed and continues to develop molecular markers that are capable of detecting genes that control the synthesis of oil, expression of yellow seedcoats and expression of resistance to blackleg. There are multiple genes controlling each of these traits and the inheritance of some of these traits is recessive. These attributes make the selection for improved breeding lines difficult under changing environmental conditions and with limited resources to examine very large segregating populations.

Marker-assisted breeding allows for the selection of seedlings with the best genetic potential to express the desired traits resulting in fewer plants or lines being evaluated in the field. There is potential to stack or pyramid multiple blackleg disease resistance genes in one line. This task can only be accomplished with the use of molecular markers since the expressed disease resistance from different sources may be indistinguishable.

The research accomplished in the previous progress reports has been summarized for the final report in Table 1. Because of the support received from ADF we have been able to identify and refine markers for at least two blackleg disease resistance genes and have identified markers associated with three genes that are essential for the expression of a good yellow seedcoat colour. Implementation of the markers will proceed in the 2001 AAFC-SRC *B. napus* breeding program and we will continue to identify, refine and validate the markers for oil content, seedcoat colour and blackleg resistance.

Technical Report

GERMPLASM

Population development: The data collected from the 2000 field season will be used to identify new genetic loci controlling yellow seed colour or to correlate with previous data on the yellow seed colour trait. The lines will also be evaluated for their potential as parental material in the development of elite germplasm. To collect a second year of data the single seed descent (SSD) population is being increased and will be planted as F8 rows in the 2001 field season. The SSD cross has a different source of yellow seed coat colour than the doubled haploid (DH) population used to develop the yellow seed molecular markers. The SSD cross will provide an additional population to test the versatility of the markers tagging yellow seed colour. It may also provide a population to develop markers tagging this additional source of yellow seed. This may become important when attempting to pyramid genes for the development of germplasm with increased yellow seed colour.

The populations were field evaluated in the final year of this project, but will also continue to be evaluated by AAFC-SRC in subsequent field seasons. The lines will also be evaluated for their potential as parental material in the development of elite germplasm.

TRAITS / MARKERS

Oil content:
We are still proceeding with the development of a molecular map to tag the oil QTLs. Because of the increase in time that it would take to develop a map using microsatellite markers with our existing equipment we have chosen to use the Amplified Fragment Length Polymorphism (AFLP) technique. If it is necessary in the future to increase the versatility of the oil content molecular markers through the use of microsatellite markers we are including in the screening of AFLP markers a number of the parents used to construct the AAFC-SRC microsatellite maps. This step will ease the alignment between the maps, if comparative mapping is required.

While preparing for the development of an AFLP map to identify molecular markers linked to oil content QTLs, we are attempting to clone candidate genes believed to play a regulatory role in lipid synthesis. Cloning the genes from the parental material responsible for the segregating oil content will allow us to identify unconserved nucleotide differences between the parents. These differences will be used to create molecular markers distinguishing the phenotypes for high and low oil content.

Blackleg resistance: The robust, PCR-based allele specific blackleg markers continue to prove useful in their ability to tag blackleg resistance in various AAFC breeding lines. Because of their usefulness the markers have challenged our lab to develop new techniques or modify existing techniques to meet the demand of screening large populations required by plant breeders.

Yellow seedcoat colour: Because of the lack of versatility of the molecular markers developed in this project to tag QTLs for yellow seed colour in other crosses microsatellite markers were attempted to be identified which showed synteny for the QTLs. To do this the linkage groups of our AFLP map and the AAFC-SRC microsattelite map needed to be aligned. This was done by comparing AFLP marker data that was available for both maps. Nearly half of the linkage groups were aligned between the maps. Unfortunately the linkage groups identified in the AFLP map that contained the yellow seed colour QTLs were not aligned with the microsatellite linkage groups. A further attempt to align the linkage groups of interest between the two maps occurred with the use of the allele specific amplicons (ASA) which are believed to flank the yellow seed colour QTLs to be used as probes in a technique known as restriction fragment length polymorphism (RFLP). These ASA will be used as probes to screen the segregating population used to develop the microsatellite maps and identify to which linkage groups these ASA probes map.

In an attempt to develop markers with increased versatility the AFLP map tagging three yellow seed coat QTLs are being integrated with the *B. napus* microsatellite map developed at AAFC-SRC by Dr. Andrew Sharpe's group. Microsatellite markers will be selected which show synteny to the yellow seed colour QTLs. It is understood that the microsatellite markers will have a higher likelihood for selecting alleles for yellow seed in differing backgrounds. We will be validating these results with segregation data from the populations field tested in 2000.

Nearly half of the linkage groups have been aligned between the AFLP map (identifying 3 QTLs for yellow seed coat colour) and Dr. Sharpe's AAFC-SRC microsatellite map. Once the alignment of the linkage groups possessing the yellow seed colour QTLs is complete the microsatellite markers showing synteny to the QTLs can be selected as tags for yellow seed coat colour.

GENERAL COMMENTS

The SSD population is to be evaluated as F8 rows in the 2001 field year. We are now actively searching for genes controlling oil content in *B. napus*. In addition to cloning oil content genes data is being produced to develop the AFLP map. The blackleg molecular markers remain essential in efficiently advancing resistant lines through AAFC-SRCs breeding programs. The AAFC-SRC breeding program is pursuing agronomic improvement of the yellow-seeded lines, and the microsatellite markers should play a role in reselecting the potential yellow seeded lines in segregating populations.

Project developed materials: Molecular markers for blackleg resistance have been and continue to be successfully used in AAFC-SRC breeding programs. Yellow seed markers exist but they possess limited versatility when used to screen for yellow seedcoat colour in different backgrounds. The SSD population is being increased to obtain enough seed to plant F8 replicated rows in the 2001 field year.

Project photos: None at this time.

Acknowledgements: Public and private sector researchers often visit the AAFC-SRC and when this project is described to them, we are sure to acknowledge the support of ADF. Daryl Somers gave a review of the Oilseeds' Marker Assisted Breeding laboratories research at the Rapeseed congress September 1999 in Canberra, the research on blackleg resistance markers and yellow seed colour will be highlighted as will the support of ADF.

Table 1: A summary of the research that occurred from July 30/97- June 30/00 in the ADF-SAF funded project for the development of molecular markers for the efficient breeding of high quality, blackleg resistant, yellow-seeded *B. napus*.

	Oil Content	Blackleg Resistance	Yellow Seed Coat Colour
July 30/97 – Dec. 31/97	- seed increase for field evaluation in 1998 of a DH population segregating for oil content that will be used to develop molecular markers.	-crosses have been made producing mapping populations for the identification of molecular markers tagging the European and Australian sources of blackleg resistance.	-development of mapping populations to genetically study two sources of yellow seedcoat colour -identification of molecular markers linked to a major yellow seed colour.gene.
Jan. 01/98 – June 30/98	-replicated field trials for two DH populations segregating for oil content. -marker development delayed to obtain a second year of data.	-DH populations segregating for blackleg resistance are under development and will be evaluated in the 1999 field season.	-development of DH populations to be evaluated in the 1999 field season. -search for markers linked to additional genes controlling yellow seed colour.
July 1/98 – Dec. 31/98	-DH population was field tested and a second year of phenotypic data was collected.	-development of molecular markers tagging Cresor and Shiralee blackleg resistance.	-identification of molecular markers tagging two additional genes that control yellow seed colour.
Jan. 01/99 – June 30/99	-attempting to identify molecular markers linked to oil content QTLs using BSA. -seed from the DH population was increased for the 1999 field season.	-the blackleg resistance molecular markers have been converted into robust, PCR based, allele specific amplicons (ASA). -identification of two adult and one cotyledon blackleg resistant gene.	-attempting to convert the yellow seed colour markers into robust, ASA markers. -seed increase of a population segregating for an additional source of yellow seed colour.
July 1/99 – Dec. 31/99	-DH population segregating for oil content was in the field for the third and final year. -molecular markers identified through BSA were not linked with oil content QTLs.	-implementation of blackleg ASA molecular markers into AAFC-SRC breeding programs.	-conversion of yellow seed colour markers into ASA markers. -seed increase for the population segregating for yellow seed colour to obtain phenotypic data from an additional field season.
Jan. 01/00 – June 30/00	-construction of an AFLP map to identify molecular markers linked to oil content QTLs. -attempting to clone genes believed to be involved in oil content.	-continued extensive use of blackleg ASA molecular markers to advance blackleg resistant lines through the AAFC-SRC breeding programs.	-ASA markers unsuccessful in identifying yellow seed in different populations. -attempting to identify microsatellite markers displaying synteny to yellow seed colour ASA markers. -seed increase of a population segrgating for yellow seed colour.